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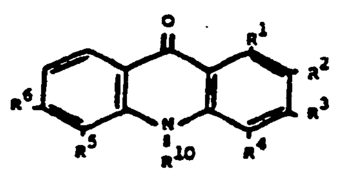

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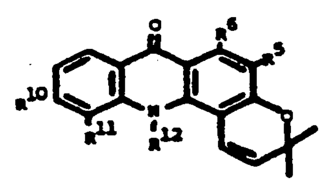

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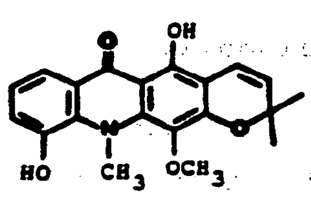

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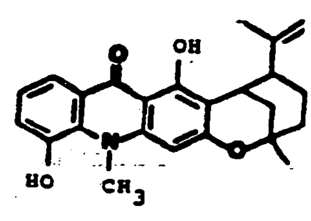

(IV)

(57) Abstract

The subject invention discloses methods of inhibiting growth of tumor cells which comprise contacting the cells with a growth inhibiting amount of a compound having one of structures: (I), wherein R^1 is a hydroxyl or acetyl group; R^2 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^3 is a hydroxyl, methoxy or acetyl group; R^4 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^5 is a hydroxyl, methoxy or acetyl group; R^6 is hydrogen or a hydroxyl, methoxy or acetyl group; and R^{10} is a methyl group; (II), wherein R^5 is hydrogen or $-CH_2-CH=C(CH_3)_2$; R^6 is a hydroxyl group; R^{10} is hydrogen or a hydroxyl group; R^{11} is a hydroxyl or methoxy group; and R^{12} is hydrogen or a methyl group (III, IV). This invention also provides compositions for inhibiting growth of tumor cells which comprise one of the above-identified compounds and wherein the compound substituents comprise hydrophilic substituents to increase solubility.

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METHODS AND COMPOSITIONS FOR INHIBITING TUMOR CELL GROWTH

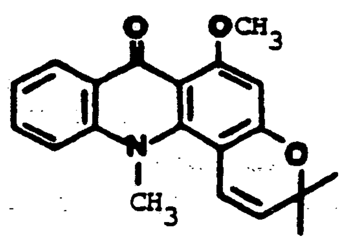
This application is a continuation-in-part of U.S. Serial No. 357,323, filed May 25, 1989, the contents of which are hereby incorporated by reference into the present disclosure.

The invention described herein was made in the course of work under Grant Nos. CA18856, CA27569 and AI26056 from the National Cancer Institute, U.S. Department of Health and Human Services. The U.S. Government may have certain rights in this invention.

Background of the Invention

A number of naturally occurring acridone alkaloids have been isolated from the family Rutaceae, but only acronycine (1) has been noted for its antitumor activity. (Throughout the specification, compounds will be cross-referenced with underlined numbers to facilitate reading and to avoid repetition of long chemical names).

1



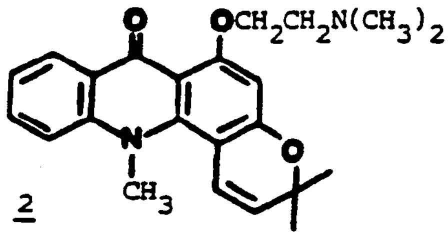
Acronycine was isolated from the bark of the Australian scrub, *Acronychi bauери Schott* (Rutaceae) (Hughes, G.K., et al., Nature, 162:223-224 (1948); McDonald, P.L. and A.V. Robertson, Aust. J. Chem., 19:275-281 (1966); Svoboda, G.H., Lloydia, 29:206-224 (1966)). Experimental studies on acronycine in animals (Finkelstein, T.Z., et al. Cancer

Chemother. Reports, 59:975-983 (1975); Schneider, J., et al., J. Med. Chem., 15:266-270 (1972); Svoboda (1966); Svoboda, G.H., et al., J. Pharm. Sci., 55:758-768 (1966); Svoboda, G.H., U.S. Patent No. 3,985,899, issued October 12, 1976; Svoboda, G.H., U.S. Patent No. 4,309,431, issued January 5, 1982) showed it to be effective against a wide range of neoplasms. Because of its antitumor activity, it has undergone clinical trials (Division of Cancer Treatment, Program Statistics, March 30, 1970, Department HEW, Public Health Service, NIH, IND status filed for acronycine).

Acronycine was found to inhibit the incorporation of extracellular nuclosides into the DNA and RNA of cultured L5178Y cells, but did not interact with DNA (Dunn, B.P., et al. Cancer Res., 33:2310-2319 (1973)). The inhibitory effect of the alkaloid may be the result of an alteration in the transport of uridine through the plasma membrane instead of impaired nucleoside or nucleotide phosphorylation (Dunn, B.P., et al. Cancer Res., 33:2310-2319 (1973)). Similar mechanisms of action were also observed in other studies which indicated that the alkaloid acts primarily on membranous organelles, and its delayed effects may be in part due to interference with the structure, function, and/or turnover of cell-surface components (Tan, P., and N. Auersperg, Cancer Res., 33:2320-2329 (1973)).

Several derivatives of acridinone have been synthesized and tested for their antitumor activity (Liska, K.J., J. Med. Chem., 15:1177-1179 (1972); Radzikowski, C., et al. Arch. Immunol. Ther. Exp., 19:219-228 (1971); Reisch, J. and S.M.E. Aly, Arch. Pharm., 319:25-28 (1986); Schneider, J., et al. J. Med. Chem. 15:266-270 (1972); Svoboda, G.H. Lloydia 29:206-224 (1966)). Of those derivatives synthesized, only *O*-(dimethylaminoethyl)noracronycine (2) (Reisch, J. and S.M.E. Aly, Arch. Pharm. 319:25-28 (1986)) and 1-nitro-N^{10}-substituted acridine-9-ones (3)

(Radzikowski, C., et al., Arch. Immunol. Ther. Exp. 19:219-228 (1971)), showed biological activity. Compound 2, bearing a charged dimethyl-aminoethyl side-chain, was reported to exhibit significant antitumor activity (Reisch, J. and S.M.E. Aly, Arch. Pharm. 319:25-28 (1986)). It is possible, therefore, that change of the overall molecular lipophilic-hydrophilic balance and electronic distribution caused by addition of a charged side-chain or nitro function on the molecule of acridine-9-one alter biological activity.



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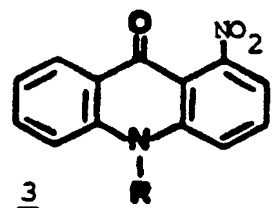


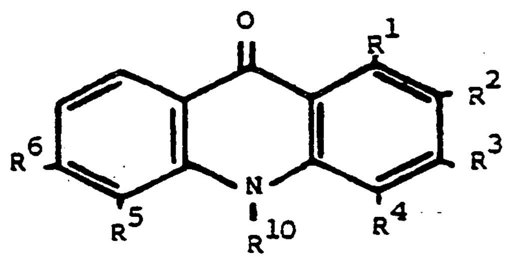
3

Svoboda, G.H., U.S. Patent Nos. 4,309,431 and 3,985,899, filed January 5, 1982 and October 12, 1976, respectively, teach a method of inhibiting growth of tumor cells using acronycine. However, they do not teach or suggest that other acridone alkaloids may be used to inhibit tumor growth.

To determine structure-activity relationships, the effects of 50 acridone alkaloids on the incorporation of the labeled precursors, [^{3}H-methyl]dThd, [5-^{3}H]Cyd and [2,3,4,5-^{3}H)L-Leu, into DNA, RNA and protein, respectively, were studied. The inhibition by these acridone alkaloids against human leukemic HL-60 cell growth was also studied.

Summary of the Invention

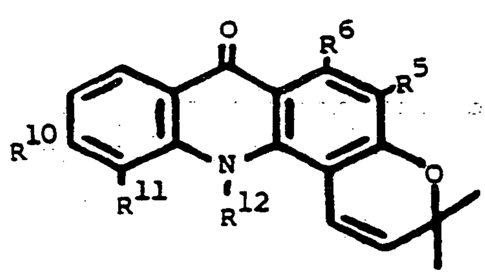
The subject invention discloses a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



wherein R^1 is a hydroxyl or acetyl group; R^2 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^3 is a hydroxyl, methoxy or acetyl group; R^4 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^5 is a hydroxyl, methoxy or acetyl group; R^6 is hydrogen or a hydroxyl, methoxy or acetyl group; and R^{10} is a methyl group.

A composition for inhibiting growth of tumor cells comprising an amount of the above-identified structure effective to inhibit growth of tumor cells and a physiologically acceptable carrier is also provided.

This invention also provides a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:

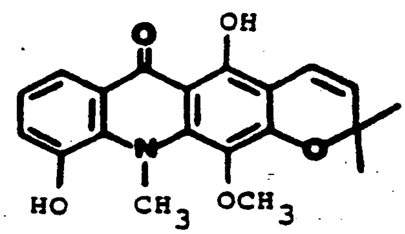


wherein R^5 is hydrogen or $-CH_2-CH=C(CH_3)_2$; R^6 is a hydroxyl group; R^{10} is hydrogen or a hydroxyl group;

R^{11} is a hydroxyl or methoxy group; and R^{12} is hydrogen or a methyl group.

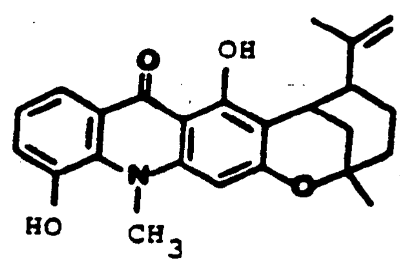
A composition for inhibiting growth of tumor cells comprising an amount of the above-identified structure effective to inhibit growth of tumor cells and a physiologically acceptable carrier is also provided.

The subject invention also discloses a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



Further provided is a composition comprising an amount of the above-identified compound effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

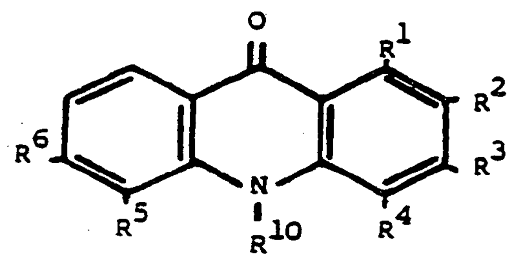
The subject invention additionally discloses a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



Further provided is a composition comprising an amount of the above-identified compound effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

Detailed Description of the Invention

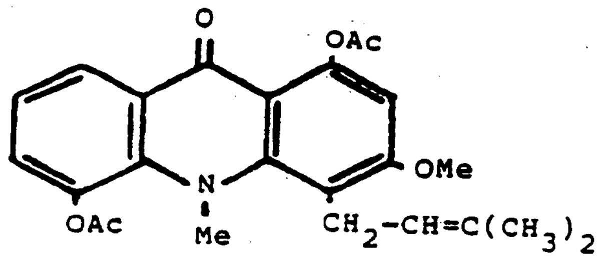
The subject invention discloses a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



wherein R^1 is a hydroxyl or acetyl group; R^2 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^3 is a hydroxyl, methoxy or acetyl group; R^4 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^5 is a hydroxyl, methoxy or acetyl group; R^6 is hydrogen or a hydroxyl, methoxy or acetyl group; and R^{10} is a methyl group.

All methods disclosed in this application may be effected _in vitro_ or _in vivo_. When these methods are performed _in vivo_, the administration of the compound may be effected by any of the well known methods, including but not limited to oral, intravenous, intramuscular, and subcutaneous. The method of delivery, the amount to be used, and the frequency of delivery are expected to vary according to the situations, the carrier used, and result desired. However, those variables are readily determinable by one skilled in the art.

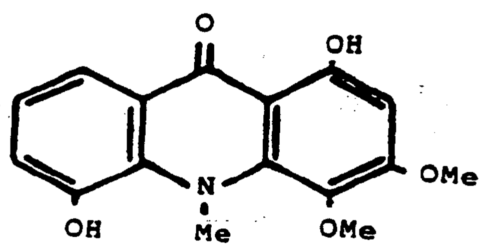
In one compound of the preferred embodiment, R^1 is an acetyl group; R^2 is hydrogen; R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is an acetyl group; and R^6 is hydrogen. This compound is O,O-diacetyl-glycocitrine-I (_14_).



O,O-Diacetyl-glycocitrine-I (<u>14</u>)

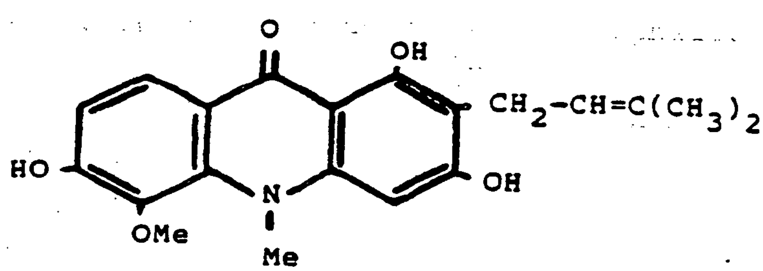
In the first subgroup of the preferred embodiment, R^1 is a hydroxyl group and R^5 is a hydroxyl or a methoxy group.

In one preferred compound of the first subgroup, R^2 is hydrogen; R^3 is a methoxy group; R^4 is a methoxy group; R^5 is a hydroxyl group; and R^6 is hydrogen. This compound is citrusinine-I (<u>16</u>)



Citrusinine-I (<u>16</u>)

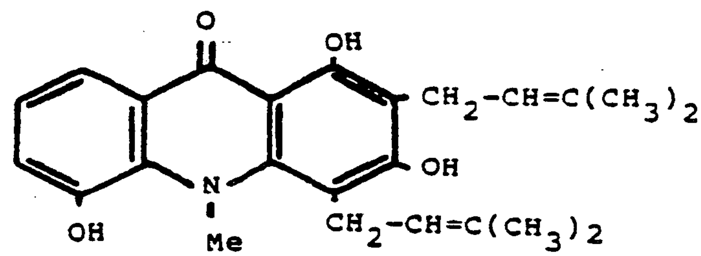
In another preferred compound of this subgroup, R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is hydrogen; R^5 is a methoxy group; and R^6 is a hydroxyl group. This compound is buntanine (<u>25</u>).



Buntanine (<u>25</u>)

The first subgroup may be further divided to a second subgroup wherein R^4 is $-CH_2-CH=C(CH_3)_2$ or a methoxy group.

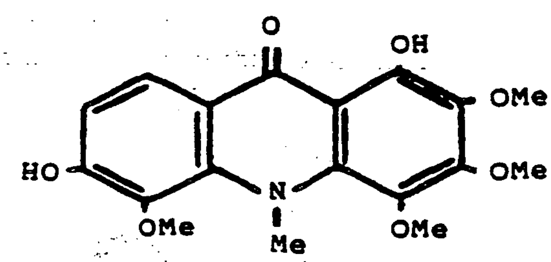
In one preferred compound of this second subgroup, R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen. This compound is N-methyl-atalaphylline (26).



N-Methyl-atalaphylline (26)

The second subgroup may be further divided to a third subgroup wherein R^2 is hydrogen or a methoxy group; and R^3 is a methoxy or acetyl group.

In one preferred compound of this subgroup, R^2, R^3, R^4 and R^5 are methoxy groups; and R^6 is a hydroxyl group. This compound is glyfoline (31):

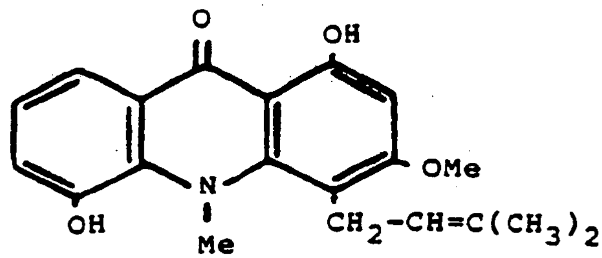


Glyfoline (31)

The third subgroup may be further divided to a fourth subgroup wherein R^2 is hydrogen.

In one preferred compound of this subgroup, R^3 is a methoxy

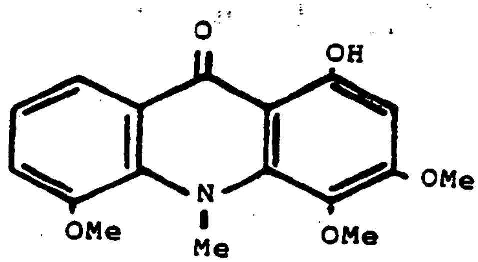
group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen. This compound is glycocitrine-I (13).



Glycocitrine-I (13)

The fourth subgroup maybe further divided to a fifth subgroup, wherein R^5 is a methoxy group.

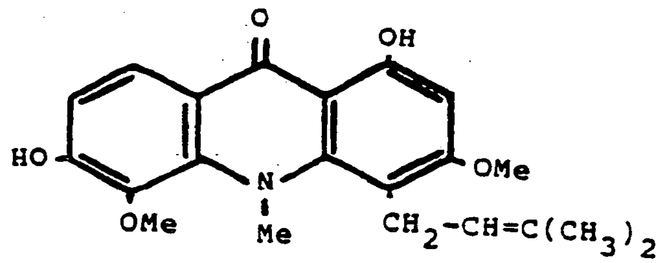
In one preferred compound of this subgroup, R^3 is methoxy group; R^4 is a methoxy group; and R^6 is hydrogen. This compound is 5-O-methyl-citrusinine-I (17):



5-O-Methyl-citrusinine-I (17)

The fifth subgroup maybe further divided to a sixth subgroup, wherein R^4 is $-CH_2-CH=C(CH_3)_2$; and R^6 is a hydroxyl, methoxy or acetyl group.

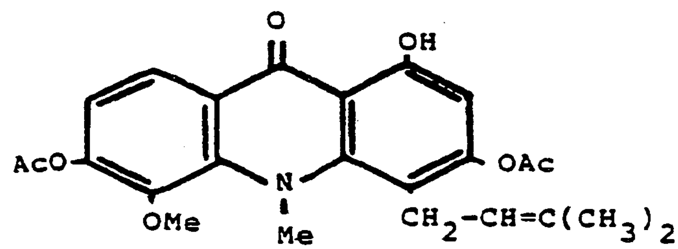
In one preferred compound of this subgroup, R^3 and R^6 are methoxy groups. This compound is grandisinine (29):



Grandisinine (29)

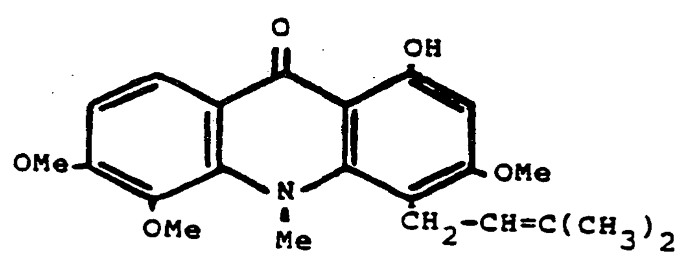
The sixth subgroup maybe further divided to a seventh subgroup, wherein R^6 is a methoxy or acetyl group.

In one preferred compound of this subgroup, R^3 is an acetyl group and R^4 is a methoxy group. This compound is 3,6-O,O-diacetyl-prenylcitpressine (28):



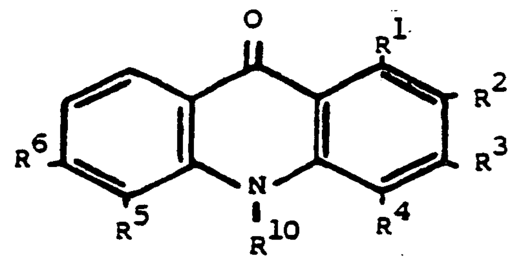
3,6-O,O-Diacetyl- (28)
prenylcitpressine

In another preferred compound of this subgroup, R^3 and R^4 are methoxy groups. This compound is balyumine-B (30):



Balyumine-B (30)

The subject invention also provides a composition for inhibiting growth of tumor cells which comprises an amount of the compound having the structure:

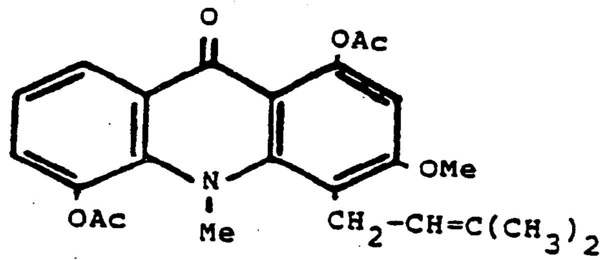


wherein R^1 is a hydroxyl or acetyl group; R^2 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^3 is a hydroxyl, methoxy or acetyl group; R^4 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^5 is a hydroxyl, methoxy or acetyl group; R^6 is hydrogen or a hydroxyl, methoxy or acetyl group; and R^{10} is a methyl group;

effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

This invention also provides for the above-indicated compositions wherein the compound comprises hydrophilic substituents to increase solubility. Hydrophilic molecules which may be used as substituents are well known in the art. However, compositions comprising the disclosed compounds with hydrophilic substituents are previously unknown.

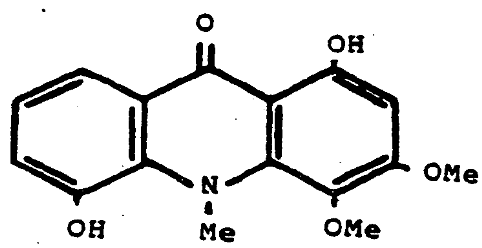
In one compound of the preferred embodiment, R^1 is an acetyl group; R^5 is hydrogen; R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is an acetyl group; and R^6 is hydrogen. This compound is O,O-diacetyl-glycocitrine-I (<u>14</u>):



O,O-Diacetyl-glycocitrine-I (14)

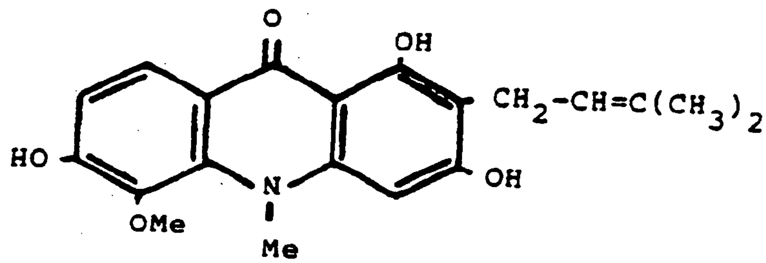
In the first subgroup of the preferred embodiment, R^1 is a hydroxyl group and R^5 is a hydroxyl or a methoxy group.

In one preferred compound of the first subgroup, R^2 is hydrogen; R^3 is a methoxy group; R^4 is a methoxy group; R^5 is a hydroxyl group; and R^6 is hydrogen. This compound is citrusinine-I (16):



Citrusinine-I (16)

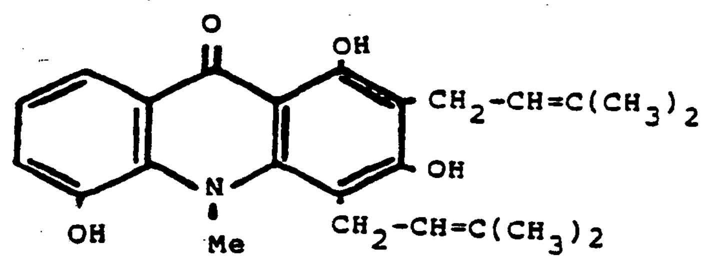
In another preferred compound of this subgroup, R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is hydrogen; R^5 is a methoxy group; and R^6 is a hydroxyl group. This compound is buntanine (25):



Buntanine (25)

The first subgroup may be further divided to a second subgroup, wherein R^4 is $-CH_2-CH=C(CH_3)_2$ or a methoxy group.

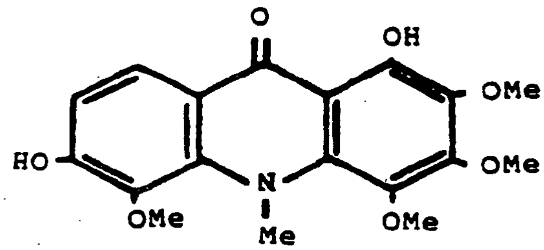
In one preferred compound of this second subgroup, R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is a hydrogen. This compound is N-methyl-atalaphylline (26):



N-Methyl-atalaphylline (26)

The second subgroup maybe further divided to a third subgroup, wherein R^2 is hydrogen or a methoxy group; and R^3 is a methoxy or acetyl group.

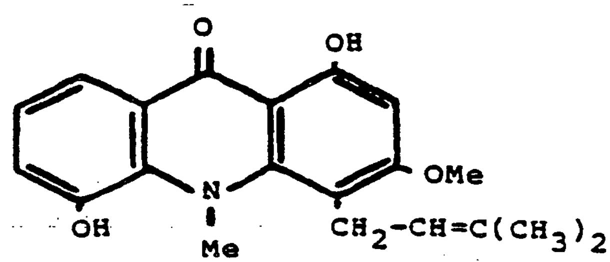
In one preferred compound of this subgroup, R^2, R^3, R^4 and R^5 are methoxy groups; and R^6 is a hydroxyl group. This compound is glyfoline (31):



Glyfoline (31)

The third subgroup maybe further divided to a fourth subgroup wherein R^2 is hydrogen.

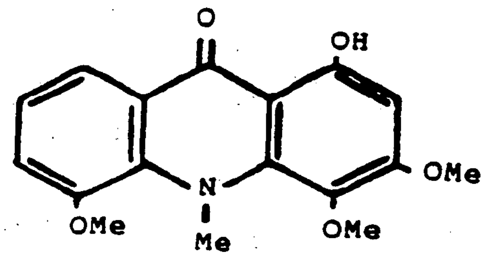
In one preferred compound of this subgroup, R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen. This compound is glycocitrine-I (13):



Glycocitrine-I (13)

The fourth subgroup maybe further divided to a fifth subgroup, wherein R^5 is a methoxy group.

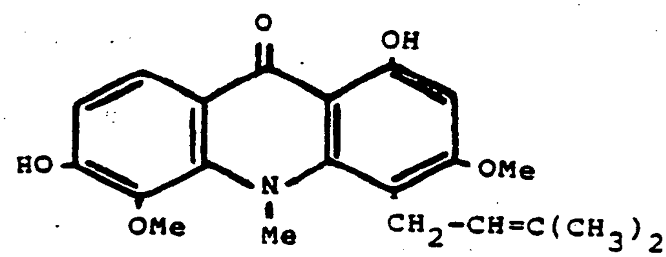
In one preferred compound of this subgroup, R^3 is a methoxy group; R^4 is a methoxy group; and R^6 is hydrogen. This compound is 5-O-methyl-citrusinine-I (17):



5-O-Methyl-citrusinine-I (17)

The fifth subgroup maybe further divided to a sixth subgroup, wherein R^4 is $-CH_2-CH=C(CH_3)_2$; and R^6 is a hydroxyl, methoxy or acetyl group.

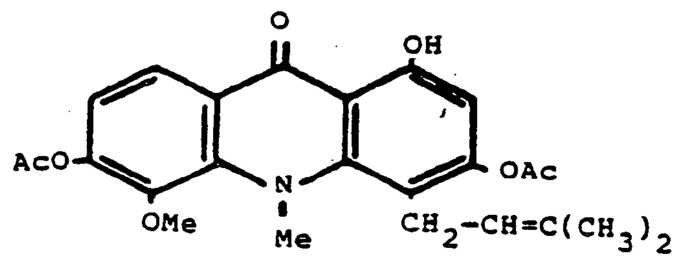
In one preferred compound of this subgroup, R^3 and R^6 are methoxy groups. This compound is grandisinine (29):



Grandisinine (29)

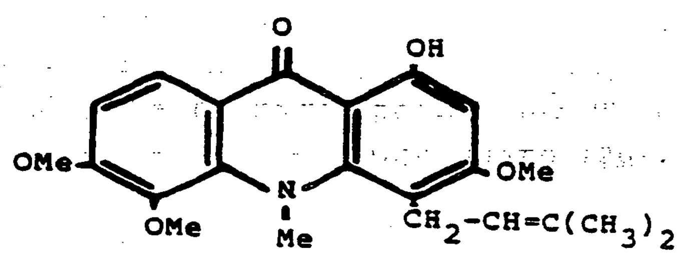
The sixth subgroup maybe further divided to a seventh subgroup, wherein R^6 is a methoxy or acetyl group.

In one preferred compound of this subgroup, R^3 is an acetyl group and R^4 is a methoxy group. This compound is 3,6-O,O-diacetyl-prenylcitpressine (28):



3,6-O,O-Diacetyl- (28)
prenylcitpressine

In another preferred compound of this subgroup, R^3 and R^4 are methoxy groups. This compound is balyumine-B (30):



Balyumine-B (30)

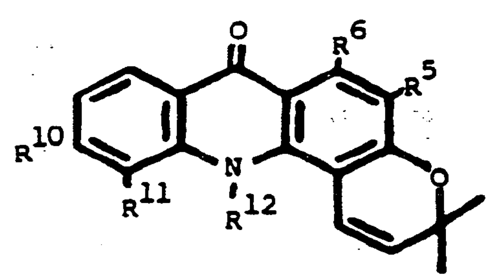
Physiologically acceptable carriers, as discussed throughout the application, are to include any carrier compatible with life. The choice of carrier is readily determinable to one skilled in the art. The physiologically acceptable carrier encompasses any of the standard carriers such as sterile solution, tablets, coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising

such carriers are formulated by well known conventional methods. However a composition comprising the compound of the subject invention effective to inhibit growth of tumor cells is previously unknown.

Any of the above-described compositions wherein the composition comprises an agent to increase solubility represent the preferred embodiment of the invention.

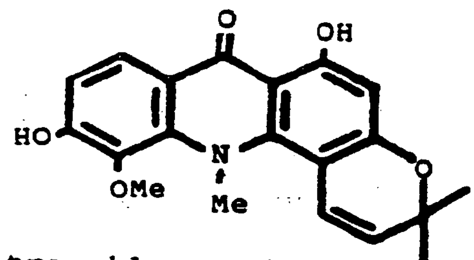
Agents to increase solubility, as used throughout the application, are to include, but are not limited to, compounds which react with the hydrophobic regions of the subject compounds. Some examples of suitable agents include Emulphor (a polyoxylethylated fatty acid which is water miscible and non-toxic when diluted 1:10 with either sterile water or sterile physiological saline solution) and polyvinylpyrrolidine. Both Emulphor and polyvinylpyrrolidine have been disclosed for use in administering acronycine by Svoboda, G.H. in U.S. patent nos. 3,985,899, filed October 12, 1976, and 4,309,431, filed January 5, 1982. However, Svoboda does not teach or suggest the use of Emulphor or polyvinylpyrrolidine in the methods or compositions of the subject invention.

This invention also provides a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



wherein R^5 is hydrogen or $-CH_2-CH=C(CH_3)_2$; R^6 is a hydroxyl group; R^{10} is a hydrogen or a hydroxyl group; R^{11} is a hydroxyl or methoxy group; and R^{12} is a hydrogen or methyl group.

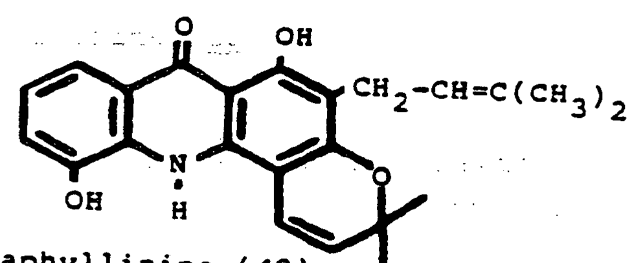
In a preferred compound of this embodiment, R^5 is hydrogen; R^{10} is a hydroxyl group; R^{11} is a methoxy group; and R^{12} is a methyl group. This compound is citracridone-I (42):



Citracridone-I (42)

The preferred embodiment may be divided to a first subgroup, wherein R^{10} is a hydrogen; and R^{11} is a hydroxyl group.

In a preferred compound of the first subgroup, R^5 is $-CH_2-CH=C(CH_3)_2$ and R^{12} is hydrogen. This compound is atalaphyllinine (40):

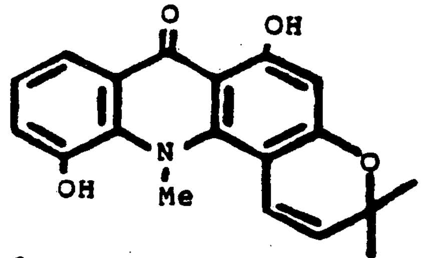


Atalaphyllinine (40)

The first subgroup may be divided to a second subgroup,

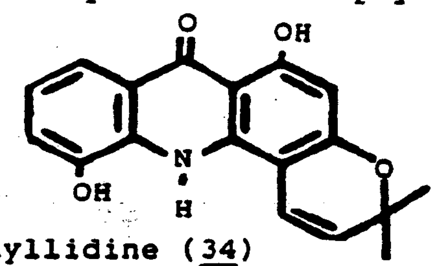
wherein R^5 is hydrogen.

In one preferred compound of the second subgroup, R^{12} is a methyl group. This compound is 11-hydroxy-noracronycine (37).



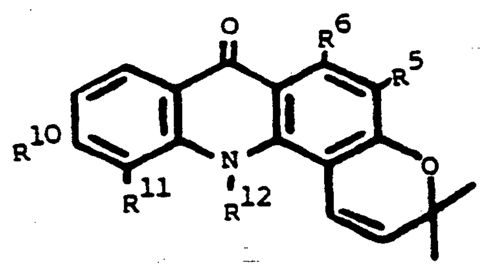
11-Hydroxy-nor- (37)
acronycine

In another preferred compound of the second subgroup, R^{12} is hydrogen. This compound is atalaphyllidine (34).



Atalaphyllidine (34)

The subjection invention further discloses a composition comprising an amount of a compound having the structure:



wherein R^5 is hydrogen or $-CH_2-CH=C(CH_3)_2$; R^6 is a hydroxyl group; R^{10} is a hydrogen or a hydroxyl group; R^{11} is a hydroxyl or methoxy group; and R^{12} is a hydrogen or methyl group;

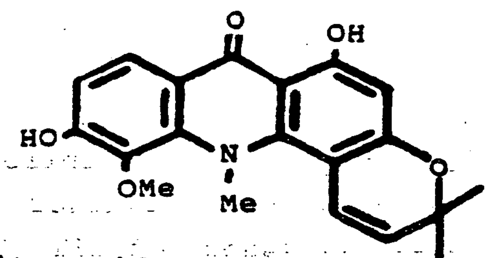
effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

This invention also provides for the above-indicated

compositions wherein the compound comprises hydrophilic substituents to increase solubility. Hydrophilic molecules which may be used as substituents are well known in the art. However, compsitions comprising the disclosed compounds with hydrophilic substituents are previously unknown.

In the preferred embodiment, R^6 is a hydroxyl group; R^{10} is hydrogen or a hydroxyl group; and R^{11} is a hydroxyl or methoxy group.

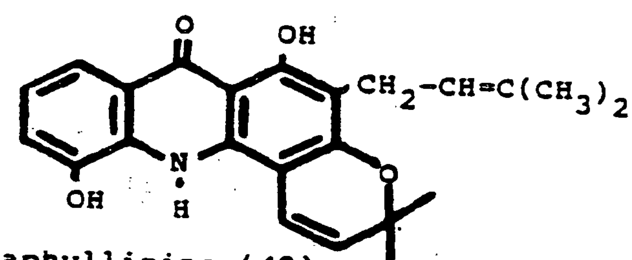
In a preferred compoind of this embodiment, R^5 is hydrogen; R10 is a hydroxyl group; R^{11} is a methoxy group; and R^{12} is a methyl group. This compound is citracridone-I (<u>42</u>):



Citracridone-I (<u>42</u>)

The preferred embodiment may be divided to a first subgroup, wherein R^{10} is a hydrogen; and R^{11} is a hydroxyl group.

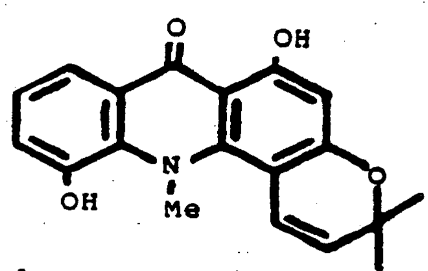
In a preferred compound of the first subgroup, R^5 is $-CH_2-CH=C(CH_3)_2$ and R^{12} is hydrogen. This compound is atalaphyllinine (<u>40</u>):



Atalaphyllinine (<u>40</u>)

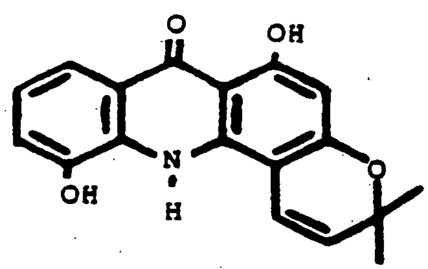
The first subgroup may be divided to a second subgroup, wherein R^5 is hydrogen.

In one preferred compound of the second subgroup, R^{12} is a methyl group. This compound is 11-hydroxy-noracronycine (37):



11-Hydroxy-nor- (37)
acronycine

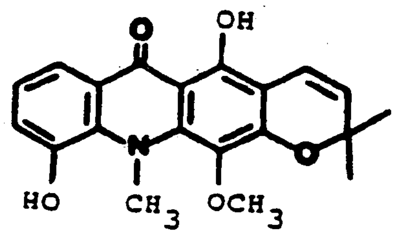
In another preferred compound of the second subgroup, R^{12} is hydrogen. This compound is atalaphyllidine (34):



Atalaphyllidine (34)

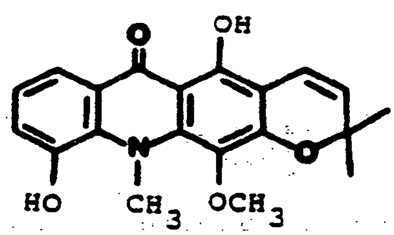
In any of the above-defined compositions, wherein the composition comprises an agent to increase solubility, it is to be considered a preferred embodiment.

The subject invention also discloses a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



Pyranofoline (48)

Further provided is a composition comprising an amount a compound having the structure:



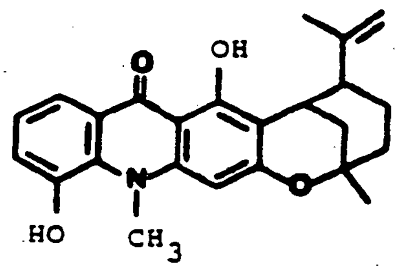
Pyranofoline (48)

effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

This invention also provides for the above-indicated compositions wherein the compound comprises hydrophilic substituents to increase solubility. Hydrophilic molecules which may be used as substituents are well known in the art. However, compositions comprising the disclosed compounds with hydrophilic substituents are previously unknown.

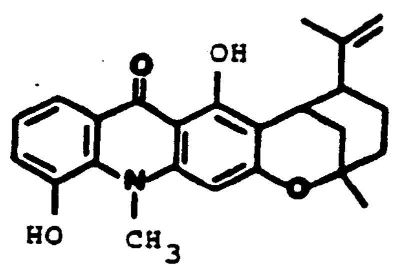
In the preferred embodiment, the composition further comprises an agent to increase solubility.

Additionally, this invention discloses a method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



Cycloglyco- (50)
foline

Also provided is a composition comprising an amount a compound having the structure:



Cycloglyco- (50)
foline

effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

This invention also provides for the above-indicated compositions wherein the compound comprises hydrophilic substituents to increase solubility. Hydrophilic molecules which may be used as substituents are well known in the art. However, compositions comprising the disclosed compounds with hydrophilic substituents are previously unknown.

Lastly, the subject invention provides the above composition wherein the composition further comprises an agent to increase solubility.

The following Experimental Detail section and Examples are set forth to aid in an understanding of the invention. These sections are not intended to, and should not be construed to, limit in any way the invention as set forth the claims which follow thereafter.

Experimental Detail

All acridone alkaloids were either previously isolated from plants indigenous to Taiwan or were obtained by partial synthesis. The isolation and structure elucidation data for each compound are referenced in Tables 1-3.

The structures of compounds 4-10 have been disclosed by Wu, T.-S., Yakugaku Zasshi, 103:1103-1107 (1983); compounds 1, 11-14, 31, 33-35, 38, 39 and 48-51 have been disclosed by Wu, T.-S., J. Chem. Soc. Perkin Trans. I., 1681-1688 (1982); compounds 15-17 and 27 have been disclosed by Wu, T.-S. and H. Furukawa, Chem. Pharm. Bull., 31:901-906 (1983); compounds 19-21, 28, and 29 have been disclosed by Wu, T.-S., C.-S. Kuoh and H. Furukawa, Phytochemistry, 22:1493-1497 (1983); compounds 22, 23, 39 and 42-45 have been disclosed by Wu, T.-S., C.-S. Kuoh and H. Furukawa, Chem. Pharm. Bull, 31:895-900 (1983); compound 25 has been disclosed by Wu, T.-S., Phytochemistry; 27, in press. (1988); compounds 26, 32, 36, 41, 42, 46 and 47 have been disclosed by Wu, T.-S. Kuoh and H. Furakawa, Phytochemistry, 21:1771-1773 (1982); compound 30 has been disclosed by Wu, T.-S., Phytochemistry, 26:871-872 (1987); and compound 52 has been disclosed by Furukawa, H., T.-S. Wu, C.-S. Kuoh, T. Sato, Y. Nagai and K. Kagei, Chem. Pharm. Bull., 32:1647-1649 (1984).

For the precursor incorporation studies, each compound (in 0.3% dimethylsulfoxide) was preincubated with HL-60 cells (a human promyelocytic leukemic cell line) 2.2 x 10^6/ml, for 15 min prior to the addition of the labeled precursor (obtained from ICN Radiochemicals, Irvine, CA) and then incubated for 30 min. The precursors used for incorporation into DNA, RNA and protein were [^{3}H-methyl]dThd (1 μCi, 0.15 nmole/ml), [5-^{3}H]Cyd (5 μCi, 0.23 nmol/ml), and [2,3,4,5-^{3}H]L-Leu (0.5 μCi, 0.004 nmol/ml), respectively. Incorporation of radioactivity in the absence of a plant compound and in the

presence of dimethylsulfoxide was used as a control. The control values for incorpation into DNA, RNA and protein were 8,500, 5,600 and 1,700 cpm/10^6 cells, respectively. The incubation conditions and the procedures for isolating DNA, RNA and protein fractions have been previously described (Chou, T.-C., et al., Cancer Res., 43:3074-3079 (1983)).

For growth inhibition studies, HL-60 cells (1.5 x 10^5/ml) were grown in RPMI 1640 media (GIBCO, Grand Island, NY) at 37°C in humidified 5% CO_2 for 72 h. Viable cells were determined using trypan blue exclusion and counted in a hemocytometer. The fractional inhibition for each compound concentration (0.0025-0.05 mg/ml in 0.1% dimethylsulfoxide) was analyzed with the median-effect plot using a computer program (Chou, T.-C and P. Talalay, Adv. Enzyme Regul., 22:27-55 (1984); Chou, J. and T.-C. Chou, Dose-Effect analysis with microcomputers: Quantitation of ED50, LD50, synergism, antagonism, low-dose risk, receptor ligand binding and enzyme kinetics. A computer software disk for Apple II series or IBM-PC and manual. Elsevier Science Publishers, Elsevier-BioSoft, Cambridge, United Kingdom (1986)). The median-effect concentration (IC_{50}) was determined automatically for three to five dose-effect levels. Cell growth in the absence of plant compound and in the presence of dimethylsulfoxide was used as a control. Dimethylsulfoxide alone inhibited cell growth 3.9% ± 1.5% during the 72 h incubation period. Those data with negative values represent activations.

<u>Results and Discussion</u>

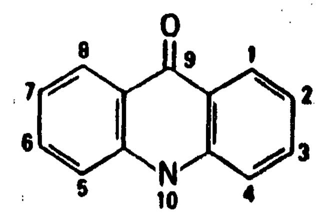
Effects of Acridine Alkaloids on Precursor Incorporation into Cellular DNA

Human promyelocytic cells (HL-60) were incubated with [^{3}H-

methyl]dThd in the presence and absence of a plant alkaloid. The percentage of inhibition of precursor incorporation in to DNA was tested with compound concentration of 0.03 mg/ml.

Acridin-9-one derivatives (Table 1), which bear 1,3-di (4 and 5) and 1,3,10-tri-sustituents (6-10) showed no significant activity on the inhibition of DNA synthesis with the exception of compound 10 which exhibited excellent inhibitory effect by inhibiting over 90% of DNA synthesis. Glycocitrine-II (11) is a potent inhibitor of cell DNA synthesis, while its 3-methyl derivative (12) shows only moderate inhibitory activity.

Table 1. Inhibition of macromolecule biosynthesis and HL-60 cell growth by acridin-9-one derivatives



No.		Position							Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/mL			Cell Growth Inhibition, IC_{50}	
		1	2	3	4	5	6	10		DNA	RNA	Protein	(mg/mL)	(μM)
4	1,3-Dihydroxyacridone	OH		OH				H	227.26	76.7	50.6	62.8	0.0206	90.7
5	1,3-O,O-Diacetylacridone	OAc		OAc				H	311.34	39.7	24.9	26.9	>0.04	>129
6	1,3-Diacetyl-N-methyl acridone	OAc		OAc				Me	325.36	23.0	2.7	-12.8	>0.04	>123

Table 1 (cont'd)

No.		Position (R)							Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/ml			Cell Growth Inhibition, IC_{50}	
		1	2	3	4	5	6	10		DNA	RNA	Protein	(mg/ml)	(μM)
7	1-Hydroxy-3-methoxy N-methyl acridone	OH		OMe				Me	255.30	48.4	39.5	25.2	>0.04	>157
8	1-Methoxy-3-Hydroxy N-methyl acridone	OMe		OH				Me	255.30	53.3	51.2	25.7	>0.04	>157
9	3-O-Acetyl-1-methoxy-R-methyl acridone	OMe		OAc				Me	297.34	71.1	61.6	30.0	0.0305	102.6
10	1,3-Dimethoxy-N-methyl acridone	OMe		OMe				Me	269.32	91.2	65.8	64.4	0.0092	34.2
11	Glycocitrine-II	OH		OH	R			Me	309.38	99.5	97.1	95.3	0.0082	26.5

Table 1 (cont'd)

No.	Position (R) 1	2	3	4	5	6	10	Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/ml DNA	RNA	Protein	Cell Growth Inhibition, IC_{50} (mg/ml)	(μM)
12 3-O-Methylglycocitrine-II	OH		OMe	R			Me	323.40	61.8	53.8	50.3	>0.04	>124
13 Glycocitrine-I	H		OMe	R	OH		Me	339.40	97.5	43.9	41.1	0.0014	4.1
14 O-O-Diacetylglycocitrine-I	OAc		OMe	R	OAc		Me	423.48	94.7	63.8	89.8	0.0056	13.2
15 Citrusinine-II	OH		OH	OMe	OH		Me	287.30	88.5	91.7	54.3	0.017	59.2
16 Citrusinine-I	OH		OMe	OMe	OH		Me	301.32	97.0	96.5	87.0	0.0026	8.6
17 5-O-Methylcitrusinine-I	OH		OMe	OMe	OMe		Me	315.34	93.4	96.4	93.0	0.0027	8.6
18 1,3,4,5-Tetramethoxy-N-methyl acridone	OMe		OMe	OMe	OMe		Me	329.36	91.5	57.0	71.8	0.0133	40.4

Table 1 (cont'd)

No.	Position (R)							Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/ml			Cell Growth Inhibition, IC_{50} (mg/ml)	(μM)
	1	2	3	4	5	6	10		DNA	RNA	Protein		
19 Grandisine-II	OH		OH		OMe	OMe	Me	301.32	76.5	96.0	27.3	>0.04	>133
20 O-Acetyl-grandisine-II	OH		OAc		OMe	OMe	Me	343.36	24.4	77.0	-5.6	0.0387	112.7
21 Grandisine-I	OH		OMe		OH	OMe	Me	301.32	71.7	69.5	22.8	>0.04	>133
22 Citpressine-I	OH		OMe		OMe	OMe	Me	301.32	65.0	80.9	90.5	>0.04	>133
23 Citpressine-II	OH		OMe		OMe	OMe	Me	315.34	34.8	95.0	28.3	>0.04	>127
24 1-O-Methyl-citpressine-II	OMe		OMe		OMe	OMe	Me	329.36	93.1	67.3	75.6	0.022	66.8
25 Buntanine	OH	R	OH		OMe	OH	Me	355.40	96.6	29.8	40.1	0.0036	10.1
26 N-Methyl-atalaphylline	OH	R	OH	R	OH		Me	393.48	99.6	28.2	44.3	0.0054	13.7
27 Prenyl-citpressine	OH		OH	R	OMe	OH	Me	355.40	52.4	21.1	-15.7	0.036	94.5

Table 1 (cont'd)

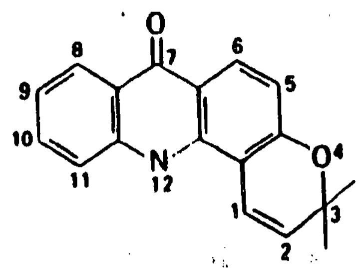
No.	Position (R)							Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/ml			Cell Growth Inhibition, IC_{50}	
	1	2	3	4	5	6	10		DNA	RNA	Protein	(mg/ml)	(µM)
28 3,6-O,O-Diacetyl-prenyl-citpres-sine	OH		OAc	R	OMe	OAc	Me	439.48	96.8	96.1	89.6	0.0022	5.0
29 Grandi-sinine	OH		OMe	R	OMe	OH	Me	355.42	98.5	96.5	94.6	0.006	16.9
30 Balyu-mine-B	OH		OMe	R	OMe	OMe	Me	383.44	86.9	92.5	87.0	0.0017	4.4
31 Glyfo-line	OH	OMe	OMe	OMe	OMe	OH	Me	361.36	93.5	65.7	90.7	0.0004	1.1

Where R = $-CH_2-CH=C(CH_3)_2$

Compounds with penta-substituents split into two categories; those with substitution at position 4 (13-18) display excellent activity, whereas others possessing a substituent at position 6 (19-24) show relatively weaker activity. The only exception is compound 24 which inhibits 93% of DNA synthesis. In general, polysubstituted acridine-9-ones (25-31) inhibit DNA synthesis.

In the series of acronycine derivatives (Table 2), 6-hydroxypyrano[2,3-c]acridine-7-one (32) did not show good activity in this study, while the inhibitory activity of its O-methylated product (33) was markedly increased. Acronycine (1) was found to inhibit about 90% of prelabeled precursor incorporation into DNA, while poor activity for its disubstituted analogs (34-36) was observed. With the exception of compound 41 which has no substituent at C-11, trisubstituted acronycine derivatives (37-40) showed potent inhibitory effects on DNA synthesis.

Table 2. Inhibition of macromolecule biosynthesis and HL-60 cell growth by acronycine derivatives



No.	Position					Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/mL			Cell Growth Inhibition, IC_{50} (mg/mL)	(μM)
	5	6	10	11	12		DNA	RNA	Protein		
32 des-N-methyl-nor-acronycine		OH			H	293.36	69.2	77.5	85.9	0.0091	31.0
33 des-N-methyl acronycine		OMe			H	307.38	93.8	67.3	86.4	0.0054	17.6
34 Atalaphyllidine		OH		OH	H	309.36	78.9	94.8	82.9	0.0013	4.2
35 Noracronycine		OH			Me	307.38	25.5	94.0	4.7	>0.04	>130
1 Acronycine		OMe			Me	293.36	91.4	21.3	25.4	0.0077	26.2
36 Severifoline	R	OH			H	361.46	20.0	ND	ND	>0.04	>111

Table 2 (cont'd)

No.	Position (R) 5	6	10	11	12	Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/ml DNA	RNA	Protein	Cell Growth Inhibition, IC_{50} (mg/ml)	(μM)
37 11-Hydroxy-noracronycine		OH		OH	Me	323.38	98.6	96.6	95.3	0.0023	7.1
38 1,2-Dihydro-11-hydroxy-nor acronycine		OH		OH	Me	325.38	98.8	76.6	92.2	0.0055	16.9
39 11-methoxy-nor-acronycine		OMe		OMe	Me	337.40	98.9	39.9	36.2	0.0058	17.2
40 Atalaphylline	R	OH		OH	H	377.46	98.8	24.4	41.8	0.0012	3.2
41 N-Methylseverifoline	R	OH			Me	375.48	21.3	-17.9	13.2	>0.04	>107
42 Citracridone-I		OH	OH	OMe	Me	353.40	98.5	96.9	90.5	0.0029	8.2

Table 2 (cont'd)

No.	Position (R)					Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/ml			Cell Growth Inhibition, IC_{50}	
	5	6	10	11	12		DNA	RNA	Protein	(mg/ml)	(μM)
43 1,2-dihydrocitracridone-I		OH	OH	OMe	Me	355.40	94.9	68.0	81.9	0.0064	18.0
44 Citracridone-II		OH	OMe	OMe	Me	367.42	77.6	96.4	62.7	>0.04	>109
45 6-O-Methylcitracridone-II		OMe	OMe	OMe	Me	381.44	98.7	37.8	49.8	0.0059	15.5
46 11-Hydroxy-N-methyl severifoline	R	OH		OH	Me	391.48	97.9	16.7	4.22	0.0059	15.1
47 11-O-Acetyl-N-methyl severifoline	R	OH		OAc	Me	433.52	58.4	16.2	36.2	0.0088	20.3

Where R = $-CH_2-CH=C(CH_3)_2$

In comparing atalaphyllidine (34) with atalaphyllinine (40), the latter showed greater activity. Thus, addition of a 3-methyl-2-butenyl side-chain at the C-5 position of 34 results in increased activity. However, addition of this side-chain at the same position in noracronycine (35) had no effect on the inhibitory activity of 41. Compounds with four substituents at 6, 10, 11 and 12 (42-45), and 5, 6, 11 and 12 (46) also exhibited significant activity. It should also be noted here that 1,2-dihydro derivatives 38 and 43 were shown to have the same potency as their parent compounds, 37 and 42, respectively.

Both derivatives of pyrano[3,2-b]acridine-6-one (48 and 49) and their analog, cycloglycofoline (50) inhibited DNA synthesis significantly, whereas furofoline derivative 51 and acridine dimer (52) were not active (Table 3).

Table 3. Inhibition of macromolecule biosynthesis and HL-60 cell growth by pyranofoline and furofoline derivatives

No.	Molecular Weight	% Inhibition of Precursor Incorporation at 0.03 mg/mL			Cell Growth Inhibition, IC_{50} (mg/mL)	(μM)
48 Pyranofoline	353.40	98.8	97.2	96.4	0.0031	8.8
49 Methoxymethyl-pyranofoline	397.44	85.3	70.1	87.3	0.0187	47.1
50 Cycloglycofoline	391.48	98.7	55.7	92.1	0.0058	14.8
51 Furofoline-II	322.38	13.1	-46.3	19.5	>0.04	>124.1
52 Glycobismine-A	602.74	-27.8	-57.4	-13.2	>0.04	>66.4

Effect of Acridine Alkaloids on the Labeled Precursor Incorporation into RNA and Protein

HL-60 cells were incubated with [5-^{3}H]Cyd or [2,3,4,5-^{3}H]L-Leu in the presence and absence of alkaloids (0.03 mg/ml) in order to find the effects of the alkaloid on the incorporation of labeled precursors into RNA or protein. Most acridine alkaloids were found to have either a moderate or low inhibitory effect on RNA and protein biosynthesis. Over 90% of RNA and protein biosynthesis were inhibited by compounds 11, 16, 17, 22, 28, 29 and 30 (Table 1). The same results were obtained from some derivatives of acronycine (Table 2) and pyranofoline derivative (50, Table 3). In most cases, the amount required to inhibit precursor incorporation into DNA was less than that to inhibit precursor incorporation into RNA or protein (i.e. DNA synthesis is inhibited most potently).

Effect of Acridine Alkaloids on the Growth of Leukemic HL-60 Cells

The plant alkaloids were further examined for their effects on cell growth inhibition (Tables 1-3).

Compounds listed in Table 1 (acridin-9-one derivatives) with substituents at 1, 3 and 10 (4-9) and 1, 3, 5, 6 and 10 (19-24), in general, did not inhibit cell growth (with the exception of compound 10). Most compounds bearing substituents at the 1, 3, 4, 5 and 10 positions (13-17, 26-31) showed significant inhibition. The most potent compound in this series was glyfoline (31) with an IC_{50} of 0.0004 mg/ml (1.11 μM).

Cell growth inhibition by pyrano[2,3-c]acridine-7-one derivatives was compared to acronycine (1) (Chou, T.-C., et

al., Cancer Res. 43:3074-3079 (1983)) (Table 2). The simple 6-hydroxyl substituted derivatives (32, 35 and 44) only showed moderate cell growth inhibitory activity but their *O*-methylated counterparts (33, 1 and 45, respectively) were found to be significantly active. Atalaphyllidine (34) and its substituted 2-(3-methyl-2-butenyl) or *N*-methyl derivatives (40 and 37) were shown to have comparable activity. Severifoline (36) was reported to have no antitumor activity, but the antitumor activity was induced significantly by introduction of an OH group at position 11 (40). A similar trend was observed on several pairs of compounds (32 vs. 34; 35 vs. 37; 41 vs. 46). The 1,2-dihydro derivatives (38 and 43) were found to be only half as potent as their parent compounds (37 and 42, respectively). A linear isomer of acronycine, pyranofoline (48) (Table 3) and its analog cycloglycofoline exhibited better activity than that of acronycine. It is also interesting to note that, in general, there is a correlation between the potency of inhibiting precursor incorporation into DNA and the potency of inhibiting cell growth among these analogs. The correlation coefficient (r) for percent inhibition of DNA synthesis (x) vs log (IC_{50} in mg/ml) for HL-60 cell growth (y) was r=0.54, whereas similar plots for RNA synthesis gave r=0.18 and for protein synthesis, r=0.43, (n=35). It appears that DNA synthesis is the major target for this group of compounds.

In summary, acronycine (1) has been shown to have a broad spectrum of antitumor activity against experimental neoplasms in laboratory animals (Table 4). In an attempt to search for more potent compounds and to determine structure-activity relationships, 50 acronycine-related acridine alkaloids were examined for their effects on inhibition of precursor incorpation into DNA, RNA and protein as well as their potency on inhibition of leukemic HL-60 cell growth.

Table 4. Experimental Tumor Spectrum of Acronycine (Svoboda et al.)

Tumor	Dose (ip, mg/kg/day)	Average weight change (g. T/C)	Average life span (days, T/C)	Percent activity
B82 leukemia	37.5 X 1 X 7	-1.4/+0.4	23.7/14.6	61
C1498 leukemia	28 X 1 X 10	-1.4/+0.8	31.5/17.6	79(7)
P1534 leukemia	30 X 1 X 10	-0.2/-0.7	16.5/18.2	0
L5178Y leukemia	28 X 1 X 10	+2.2/+3.2	24.2/15.0	62
AKR leukemia	28 X 1 X 10	+0.1/+0.8	38.3/21.5	78(5)
Ehrlich ascites	30 X 1 X 10	+5.6/+7.8	21.8/18.4	0(1)
			Average tumor size (mm, T/C)	
Sarcoma 180	30 X 1 X 10	+3.2/+6.0	7.1/11.9	40(9)
Mecca lymphosarcoma	30 X 1 X 10	-0.4/+2.5	6.2/16.9	63(7)
Ridgeway osteogenic sarcoma	48 X 1 X 9	-0.6/+3.4	0/9.6	100(10)
X5563 myeloma	30 X 1 X 8	+0.1/+0.3	0/9.1	100(8)
Adrenocarcinoma 755	30 X 1 X 10	-0.5/+1.9	11.9/19.7	40(10)
Shionogi carcinoma 115	36 X 1 X 9	+1.4/+1.4	0/15.3	100(7)
S91 melanoma	36 X 1 X 9	-1.4/+0.1	0/14.1	100(4)

In general, derivatives of acronycine (pyrano[2,3-c]acridin-7-one) (Table 2) exhibited the most potent inhibition on the growth of leukemic HL-60 cells. It has also been found that acridin-9-one derivatives (Table 1) with sustituents at the 1, 3, 4 and 10 positions (13, 14, 16, 17, 26, 28, 29 and 31) had significant activity against leukemic cells *in vitro*. There are structural similarities between these compounds and acronycine; the latter is considered as a 1, 3, 4 and 10 tetra-substituted acridin-9-one derivative. Accordingly, substituents at the above four positions of the acridin-9-one ring system may be essential for optimum biological activity.

The above structure-activity relationships may provide useful directions for future synthetic approaches to developing new antitumor chemotherapeutic agents.

Summary of In Vivo Preliminary Studies on Acridones (Tables 5-7)

A. L1210 Leukemia (Natural products used)

Compound 31 (SK-32889, TSA-35, glyfoline) showed some activity against L1210 leukemia in BDF mice at a low dose (12.5 mg/kg, starting day 1 for 4 days, i.p. injection)

Compound 34 (TSA-36) also showed evidence of antileukemic activity.

Compound 37 (TSA-7) and compound 17 (TSA-15) were not active.

B. Lewis Lung Carcinoma (Synthetic compounds used)

Compound 31 at 25 and 50 mg/kg daily for 4 days i.p. injection to C57BL/6 mice showed less toxicity to mice than that of compound 1 (acronycine, TSA-33, SK 32121). At 25 and 50 mg/kg, compound 31 increased life-span by 42% and >92%, respectively, whereas compound 1 increased life-span by 40% and 40%, respectively.

There were 2 out of 5 long-term survivals for 50 mg/kg for compound 31. Other groups had no long term survivals.

C. Carcinoma E07713 (Synthetic compounds used)

At 100 mg/kg daily for 4 days i.p. compound 1 showed 1 out of 3 toxicity induced deaths but no deaths were observed as a consequence of administration of compound 31.

At 50 mg/kg and 100 mg/kg, compound 31 increased life-span by 17% and >39%, respectively, whereas compound 1 increased life-span by 26% and 4% (although toxic), respectively.

Table 5. Survival Time Evaluation
Tumor-Lewis Lung C. $3x10^5$ cells (ip) in C57BL/6, FEM, 19-21g
Drug: A=SK 32121 TSA-acronycine
B=SK 32089 TSA-35
Diluent-CMC + 1 drop Tween 80 Schedule: Day 1, QDX4 (ip)

#		Dose MG/KG	Day #7 AWC (g)	Survival Time										AST+	%ILS *	N/T
1	CONTROL		+0.1	10	10	10	10	10	10	10	11	11	12	10.4		0/10
2	Acronycin	50	-0.8	14	14	15	15	15						14.6	40	0/5
3	Acronycin	25	-0.6	14	14	14	14	17						14.6	40	0/5
4	TSA-35	50	+0.2	14	14	14	>29	>29						>20	>92	2/5
5	TSA-35	25	+0.2	14	14	14	14	18						14.8	42	0/5

* Increase in lifespan of 25% or greater indicates activity. Mice dying of toxicity (t) not included in AST evaluation. N/T = number of 29 day survivors/total mice.

\# Average weight change. Changes of less than 1 gm are not considered significant. Tumor may contribute to the body weight. Toxicity decreases body weight.

\+ Average survival time.

Table 6. Survival Time Evaluation
Tumor-Carcinoma EO771 3×10^5 cells (ip) in C57BL/6, FEM, 19-21g
Drug: A=SK 32121 TSA-acronycine
B=SK 32089 TSA-35
Diluent-CMC + 1 drop Tween 80 Schedule: Day 1, QDX4 (ip)

#		Dose MG/KG	Day #7 AWC (g)	Survival Time					AST+	%ILS *	N/T
1	CONTROL		+1.4	10	10	10	11	12	10.6		0/5
2	Acronycin	100	+0.5	4t	10	12			11.0	4	0/3
3	Acronycin	50	+0.4	11	11	14	14	17	13.4	26	0/5
4	TSA-35	100	+0.3	11	11	>22			>15	>39	1/3
5	TSA-35	50	+0.6	10	11	12	14	15	12.4	17	0/5

* Increase in lifespan of 25% or greater indicates activity. Mice dying of toxicity (t) not included in AST evaluation. N/T = number of 22 day survivors/total mice.

\# Average weight change. Changes of less than 1 gm are not considered significant. Tumor may contribute to the body weight. Toxicity decreases body weight.

\+ Average survival time.

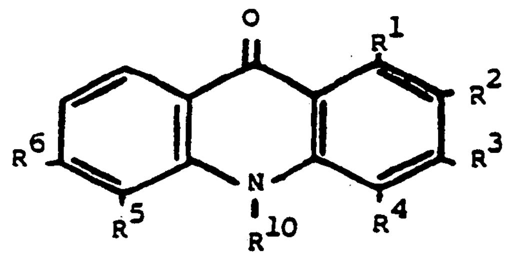
Table 7. Survival Time Evaluation
Tumor-L1210 10^6 cells (ip) in BDF1 (female) 19-21g
Drug: A=SK 32887 TSA-7
B=SK 32888 TSA-15
C=SK 32889 TSA-35
Diluent-DMSO, 0.05ml/m
Schedule: Day 1, QDX4 (ip)

#	Dose MG/KG	Day 6 AWC(g)	Survival Time	MST	%ILS*	N/T
1	Control (DMSO)	+2.6	7 7 7 7 7	7.0		0/5
2	SK 32887 6.25 11-Hydroxy-nor-acronycine (37) TSA-7	+2.0	7 7	7.0	0	0/2
3	SK 32887 25 11-Hydroxy-nor-acronycine (37) TSA-7	+1.8	7 7	7.0	0	0/2
4	SK 32888 6.25 5-O-Methyl-citrusinine-I (17) TSA-15	+1.8	7 7	7.0	0	0/2
5	SK 32888 12.5 5-O-Methyl-citrusinine-I (17) TSA-15	+1.5	7 7	7.0	0	0/2
6	SK 32889 6.25 Glyfoline (31) TSA-35	+1.8	7 7	7.0	0	0/2
7	SK 32889 12.5 Glyfoline (31) TSA-35	+2.0	8 8	8.0	14	0/2
1	SK 32090 6.25 Atalaphyllidine (34) TSA-36	+1.7	7 7	7.0	0	0/2
2	SK 32090 25. Atalaphyllidine (34) TSA-36	+1.5	7 8	7.5	7	0/2
3	SK 32091 12.5 Cycloglycofoline (50) TSA-48	+1.8	8 9	8.5	21	0/2

*Increase in lifespan of 25% or greater indicates activity.
Mice dying of toxicity (t) not included in MST evaluation.
N/T = Number of nine day survivors/total mice.

What is claimed is:

1. A method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



wherein R^1 is a hydroxyl or acetyl group; R^2 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^3 is a hydroxyl, methoxy or acetyl group; R^4 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^5 is a hydroxyl, methoxy or acetyl group; R^6 is hydrogen or a hydroxyl, methoxy or acetyl group; and R^{10} is a methyl group.

2. A method of claim 1, wherein R^1 is an acetyl group; R^2 is hydrogen; R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is an acetyl group; and R^6 is hydrogen.

3. A method of claim 1, wherein R^1 is a hydroxyl group and R^5 is a hydroxyl or a methoxy group.

4. A method of claim 3, wherein R^2 is hydrogen; R^3 is a methoxy group; R^4 is a methoxy group; R^5 is a hydroxyl group; and R^6 is hydrogen.

5. A method of claim 3, wherein R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is hydrogen; R^5 is a methoxy group; and R^6 is a hydroxyl group.

6. A method of claim 3, wherein R^4 is $-CH_2-CH=C(CH_3)_2$ or a methoxy group.

7. A method of claim 6, wherein R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen.

8. A method of claim 6, wherein R^2 is hydrogen or a methoxy group; and R^3 is a methoxy or acetyl group.

9. A method of claim 8, wherein R^2, R^3, R^4 and R^5 are methoxy groups; and R^6 is a hydroxyl group.

10. A method of claim 8, wherein R^2 is hydrogen.

11. A method of claim 10, wherein R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen.

12. A method of claim 10, wherein R^5 is a methoxy group.

13. A method of claim 12, wherein R^3 is a methoxy group; R^4 is a methoxy group; and R^6 is hydrogen.

14. A method of claim 12, wherein R^4 is $-CH_2-CH=C(CH_3)_2$; and R^6 and is a hydroxyl, methoxy or acetyl group.

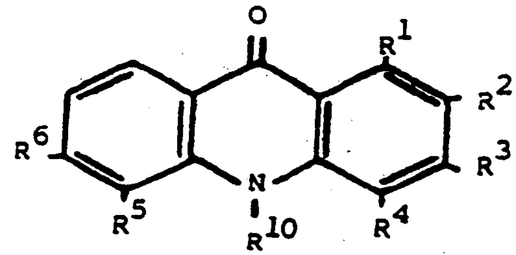
15. A method of claim 14, wherein R^3 and R^6 are methoxy groups.

16. A method of claim 14, wherein R^6 is a methoxy or acetyl group.

17. A method of claim 16, wherein R^3 is an acetyl group and R^4 is a methoxy group.

18. A method of claim 16, wherein R^3 and R^4 are methoxy groups.

19. A composition for inhibiting growth of tumor cells which comprises an amount of the compound having the structure:



wherein R^1 is a hydroxyl or acetyl group; R^2 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^3 is a hydroxyl, methoxy or acetyl group; R^4 is hydrogen, $-CH_2-CH=C(CH_3)_2$ or a methoxy group; R^5 is a hydroxyl, methoxy or acetyl group; R^6 is hydrogen or a hydroxyl, methoxy or acetyl group; and R^{10} is a methyl group;

effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

20. A composition of claim 19, wherein R^1 is an acetyl group; R^2 is hydrogen; R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is an acetyl group; and R^6 is hydrogen.

21. A composition of claim 19, wherein R^1 is a hydroxyl group and R^5 is a hydroxyl or a methoxy group.

22. A composition of claim 21, wherein R^2 is hydrogen; R^3 is a methoxy group; R^4 is a methoxy group; R^5 is a hydroxyl group; and R^6 is hydrogen.

23. A composition of claim 21, wherein R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is hydrogen; R^5 is a methoxy group; and R^6 is a hydroxyl group.

24. A composition of claim 21, wherein R^4 is $-CH_2-CH=C(CH_3)_2$ or a methoxy group.

25. A composition of claim 24, wherein R^2 is $-CH_2-CH=C(CH_3)_2$; R^3 is a hydroxyl group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen.

26. A composition of claim 24, wherein R^2 is hydrogen or a methoxy group; and R^3 is a methoxy or acetyl group.

27. A composition of claim 26, wherein R^2, R^3, R^4 and R^5 are methoxy groups; and R^6 is a hydroxyl group.

28. A composition of claim 26, wherein R^2 is hydrogen.

29. A composition of claim 28, wherein R^3 is a methoxy group; R^4 is $-CH_2-CH=C(CH_3)_2$; R^5 is a hydroxyl group; and R^6 is hydrogen.

30. A composition of claim 28, wherein R^5 is a methoxy group.

31. A composition of claim 30, wherein R^3 is a methoxy group; R^4 is a methoxy group; and R^6 is hydrogen.

32. A composition of claim 30, wherein R^4 is $-CH_2-CH=C(CH_3)_2$; and R^6 is a hydroxyl, methoxy or acetyl group.

33. A composition of claim 32, wherein R^3 and R^6 are methoxy groups.

34. A composition of claim 32, wherein R^6 is a methoxy or acetyl group.

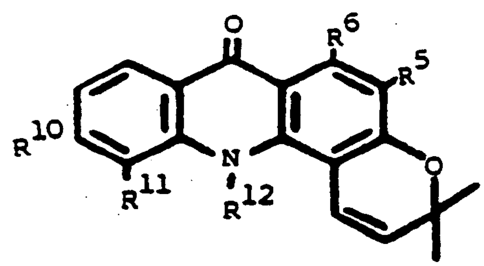
35. A composition of claim 34, wherein R^3 is an acetyl

group and R^4 is a methoxy group.

36. A composition of claim 34, wherein R^3 and R^4 are methoxy groups.

37. A composition of claim 19, wherein the compound comprises hydrophilic substituents to increase solubility.

38. A method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



wherein R^5 is hydrogen or $-CH_2-CH=C(CH_3)_2$; R^6 is a hydroxyl group; R^{10} is hydrogen or a hydroxyl group; R^{11} is a hydroxyl or methoxy group; and R^{12} is hydrogen or a methyl group;

39. A method of claim 38, wherein R^5 is hydrogen; R^{10} is a hydroxyl group; R^{11} is a methoxy group; and R^{12} is a methyl group.

40. A method of claim 38, wherein R^{10} is a hydrogen; and R^{11} is a hydroxyl group.

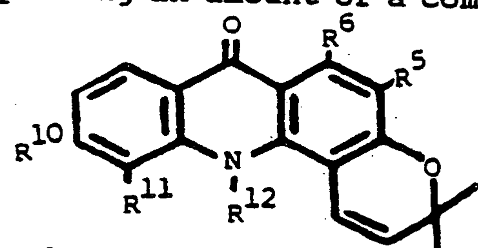
41. A method of claim 40, wherein R^5 is $-CH_2-CH=C(CH_3)_2$ and R^{12} is hydrogen.

42. A method of claim 40, wherein R^5 is hydrogen.

43. A method of claim 42, wherein R^{12} is a methyl group.

44. A method of claim 42, wherein R^{12} is hydrogen.

45. A composition comprising an amount of a compound having the structure:



wherein R^5 is hydrogen or $-CH_2-CH=C(CH_3)_2$; R^6 is a hydroxyl group; R^{10} is hydrogen or a hydroxyl group; R^{11} is a hydroxyl or methoxy group; and R^{12} is hydrogen or a methyl group;

effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

46. A composition of claim 45, wherein R^5 is hydrogen; R^{10} is a hydroxyl group; R^{11} is a methoxy group; and R^{12} is a methyl group.

47. A composition of claim 45, wherein R^{10} is a hydrogen; and R^{11} is a hydroxyl group.

48. A composition of claim 47, wherein R^5 is $-CH_2-CH=C(CH_3)_2$ and R^{12} is hydrogen.

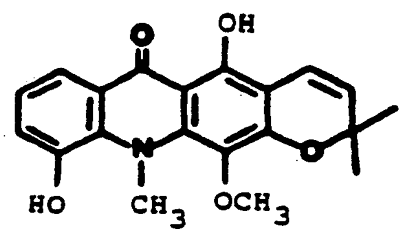
49. A composition of claim 47, wherein R^5 is hydrogen.

50. A composition of claim 49, wherein R^{12} is a methyl group.

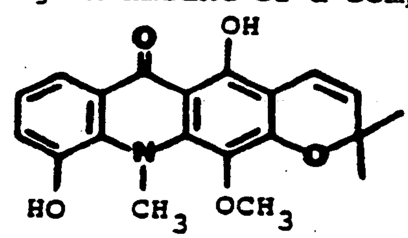
51. A composition of claim 49, wherein R^{12} is hydrogen.

52. A composition of claim 45, wherein the compound comprises hydrophilic substituents to increase solubility.

53. A method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



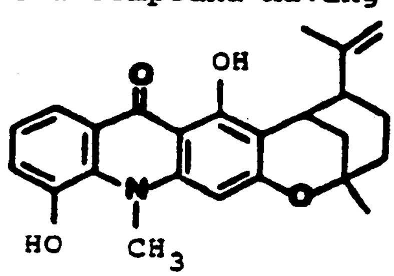
54. A composition comprising an amount of a compound having the structure:



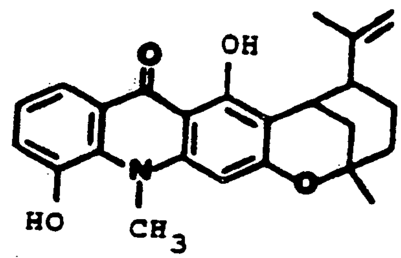
effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

55. A composition of claim 54, wherein the compound comprises hydrophilic substituents to increase solubility.

56. A method of inhibiting growth of tumor cells which comprises contacting the cells with a growth inhibiting amount of a compound having the structure:



57. A composition comprising an amount of a compound having the structure:



effective to inhibit growth of tumor cells and a physiologically acceptable carrier.

58. A composition of claim 57, wherein the compound comprises hydrophilic substituents to increase solubility.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/03065

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A01N 43/42
U.S. CL: 514/285,297

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	514/285,297

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	Chemical abstracts, volume 111, no. 14, issued 1989 (Columbus, Ohio, U.S.A.), H. Furukawa, "anticancer agents containing acridones", the abstract no. 120933 p, JP01040426A2 10 february 1989. (JP)	1-37
y	Archivum immunologies El Therapiae Experimentalis, volume 19, 1971, Radzikowski", A search for Antitumor compounds", pp 219-228	1-37
Y	Antivival Research, volume 12, April 1989, Yamamoto, "Anti-herpesvirus activity of citrusinine-I, a new acridone alkaloid, and related compounds". pages 21-36	1-37

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
25 JUNE 1990	16 AUG 1990
International Searching Authority [1]	**Signature of Authorized Officer**
ISA/US	Nguyen Ngoc-Ho For G.S. KISHORE NGUYEN NGOC-HO INTERNATIONAL DIVISION

International Application No. PCT/US90/03065

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . , because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers____________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Claims 1-37 are drawn to composition and method pertaining to species 1
Claims 38-52 are drawn to composition and method pertaining to species 2
Claims 53-55 are drawn to compositions and method pertaining to species 3
Claims 56-58 are drawn to compositions and method pertaining to species 4
The recited species are distinct invention which do not form a single inventive concept

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-37

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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